EFFECTS OF SOY ISOFLAVONES ON THE ATTAINMENT OF PUBERTY IN EWE LAMBS

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ABSTRACT

In humans, diets containing high levels of soy isoflavones have shown to increase circulating estrogen levels. With this increase, early attainment of puberty has been experienced. Although a negative in humans, this could be a substantial gain in the some species of livestock. This research is designed to determine the effects of soy isoflavones on early development and early attainment of puberty in ewe lambs. Newly born Rambouillet and Suffolk ewe lambs were randomly assigned to either the control group; cotton seed meal protein, or the treatment group; soybean meal protein. Lambs were fed treatment specific diets from just following birth until breeding. Weights and blood samples were drawn to determine growth and performance as well as serum estrogen and progesterone levels. No difference ($P > 0.05$) in growth and performance and reproductive activity was established between treatments. However, a difference was established ($P < 0.05$) between estrogen concentration.

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INTRODUCTION

Many different reproductive advances have been reached in the livestock industry. Because of these advances, factors such as genetic selection and artificial insemination have become resources the small producer now has available. One problem that producers of all sizes now face is reproductive efficiency. With sheep operations, the main goal is to yield the highest number of offspring per female possible. The most limiting factor in the reproductive success of any operation is the first year females. In the sheep industry, if a producer is able to increase the conception rate of their ewe lambs they would be able to increase productivity of the ewe's life span and in return increase revenue to the producer.

One factor that may become the key to increasing ewe lamb conception rates has shown to be a negative in humans. This issue is the attainment of puberty at a younger age. Fallon and Enig (2009) reported that 14.7 percent of Caucasian girls and almost 50 percent of African-American girls showed signs of early puberty. This early onset of puberty has been linked to soy based infant formula at which African-American girls had consumed at a higher rate. Although little research has been done to determine the effects of soy proteins on livestock, a study done by Ede et al. (2011) showed that ewes fed diets containing elevated levels of soy proteins obtained higher plasma estradiol levels than ewes on a control diet with no soy proteins.

With the information from these studies, it may be possible to increase ewe lamb reproductive activity and in return increase profits for sheep producers each year. Therefore,

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reproductive activity and in return increase profits for sheep producers each year. The objectives of this study are to determine the effects of soy isoflavones on early life development as well as determine if soy isoflavones could allow ewe lambs to become reproductively efficient at an earlier age.

OBJECTIVES

1. Determine the effects of soy isoflavones on early life development in ewe lambs from birth to post weaning
2. Determine the effects of soy isoflavones on attainment of early puberty in ewe lambs

LITERATURE REVIEW

Attainment of puberty in the ewe lamb can be defined in several ways. It can be considered as the time of first ovulation or even at what time the ewe can support pregnancy without detrimental effect. Many different factors can affect the rate individual ewe lambs reach puberty, including but not limited to genetics, threshold body size, and certain external factors (Senger, 1999). Genetics of an individual animal have shown to greatly affect the rate the animal reaches puberty. Quirke et al. (1985) showed that the first estrus in Rambouillet ewe lambs occurred later in the season and at a greater age when compared to Finn or Finn-Dorset groups. All animals were reared in the same environment and on the same nutritional plan, which suggests that genetic differences accounted for the differences in the onset of first estrus.

Another major factor that influences at what age a ewe can support pregnancy without detrimental consequences would be the threshold body size of the ewe (Senger, 1999). Threshold body size is defined as the certain body size that is required before the onset of puberty can be initiated. This is a major factor because the energy requirements that are associated with follicular development, ovulation, and ova/embryo development are not very extensive, but when considering the metabolic cost of pregnancy and lactation it becomes much more extensive (Senger, 1999). A ewe's threshold body size is a range from 50-70% of the adult body weight depending on environmental conditions as well as within and between breed factors (Dýrmundsson, 1981).

External factors probably play the largest role in reproductive development. These can include environmental conditions, social factors, and the general nutrition plan of the

operation. Environmental conditions are the least predictable of all external factors. Different social factors can influence different species in different ways. Sheep are very seasonal breeders, so season of birth can influence onset of puberty (Senger, 1999). Sheep are short-day breeders. Their breeding season is initiated as the ration of daylight to darkness decreases and ends when increasing day length reach a ratio of nearly equal daylight and darkness (Bearden et at., 2004). The most important external factor that is in complete control of the producer is a proper nutrition plan, which can drastically change at what age ewe lambs complete puberty. Ewe lambs growing at faster rates will exhibit their first estrus and are more likely to conceive at a lower age and heavier body weight than ewe lambs growing at slower rates (Dýrmundsson, 1981). Thus, proper nutrition can enable the body to reach puberty at an early age but it also follows the concept of threshold body size. On normal dietary plans accelerating nutrition can enable the ewe lamb to reach puberty at an earlier age but research has shown how certain plant derived compounds, such as phytoestrogens, can increase estrogen levels in the body and may possibly increase the rate at which ewe lambs experience puberty (Ede et. al., 2011)

Phytoestrogens are plant-derived compounds that have properties similar to estrogen. There are many subclasses of phytoestrogens but the ones found most often in dietary substances are bioflavonoids, isoflavones, flavones, and coumestans. Soy products are by far the most abundant source of these compounds. Soy isoflavones are non-steroidal compounds which are structurally similar to estradiol-17β (Ogbuewu et. al., 2010). Because isoflavones are structurally similar, they have the ability of binding to the receptor sites and mimic estradiol-17β's effects in the female. In a normal female, the body begins to release estradiol-

17β once she has reached the beginning stages of puberty. Once released, estradiol-17β stimulates the hypothalamus to release GnRH which causes the anterior pituitary to release FSH and LH. This will cause the female to respond by producing ovarian follicles and releasing estradiol (Senger, 1999). The release of the estradiol will cause the cycle to repeat itself sending the body into puberty. As levels of soy isoflavones elevate in the body, there is a possibility of falsely sending the body into premature puberty. Research has shown that this is also possible in humans (Fallon and Enig, 2009).

Fallon and Enig (2009) reported that 14.7 percent of Caucasian girls and almost 50 percent of African-American girls showed signs of early puberty in development of secondary sex characteristics. This was linked to the soy infant formulas that both consumed but at which African-American girls were fed at higher rates. Fallon and Enig (2009) then reported that the circulating concentration of isoflavones in infants fed soy-based formula were 13,000 to 22,000 times higher than plasma estradiol concentrations in infants on cow milk formula. Daniel (2004) reported that natural estrogen levels double during the first month of life, then decline and remain at low levels until puberty. With increased estrogens in the environment in the diet, an alarming number of girls are entering puberty much earlier than normal (Daniel, 2004). Daniel (2004) also reports that some girls are showing signs of puberty such as breast development or pubic hair growth before the age of three.

Although negative in humans, early onset of puberty in animals, particular livestock, could be a drastic benefit to a diminishing industry. Ede et al. (2011) fed ewe lambs three different rations containing three different levels of soy proteins, 1 (control, no soy), 2 (low soy), and 3 (high soy). Ewe lamb fed diets 2 and 3 had a substantial difference of circulating

estrogen than that of the ewes on the control diet. This suggests that the increased estrogen levels could prematurely cause the onset of puberty, as found in Fallon and Enig (2009), allowing an increase in ewe lamb conception rates. Ford et al. (2006) reported that ovariectomized gilts still maintained normal reproductive response when administered genistein, a soy isoflavones.

If high levels of soy isoflavones could cause ewe lambs to attain puberty at a younger age as seen in humans, producers could expect ewe lamb conception rates to increase. By doing so, producers' revenue would increase and diminishing sheep numbers may stabilize as a result.

The purpose of this study is to determine if feeding soy based protein early in life can increase circulating estrogen in ewe lambs to the point of mimicking natural onset of puberty, and if so would this increase ewe lamb conception rates to benefit production in the industry.

MATERIALS AND METHODS

Recently born Rambouillet and Suffolk ewe lambs were randomly assigned to one of two treatments when removed from lambing facilities at 14 d of age. Lambs were assigned randomly to a treatment by splitting ewes in an alternating fashion and placing lambs in respective treatment groups. The two treatments for the study were 1) control; cotton seed meal protein 2) treatment; soy bean meal protein. Each treatment group was placed on individual wheat fields which contained similar size plots of native rangeland, to ensure proper cover and to provide similar vegetation to each group.

Creep feeders were placed near water in each pasture containing the diet (Table 1) specific to the treatment for lambs to consume free choice. Lambs remained on creep feeders with treatment specific diets until weaning. The average daily intake of the creep feed was approximately 0.19 kg/head/d. At weaning blood samples were collected for analysis of estrogen levels. After weaning, ewe lambs were placed in feedlot type pens until breeding. Lambs had ad libitum access to water, shade, haygrazer hay, and were fed treatment specific diets on an increasing basis of 3.5% of their body weight (Table 1).

Weights were collected at weaning and at the time of ram introduction. In addition to weights, reproductive activity and development was monitored by collecting blood from the lambs. The blood was centrifuged at 2000 x g for 30 minutes to separate serum. Blood was collected using jugular veinapuncture methods into corvac serum separator tubes twice per week. Serum samples were shipped frozen to New Mexico State University's endocrinology laboratory. Serum was analyzed for progesterone concentration. Ewe lambs were considered

Table 1. Ingredient composition of diets used during the creep feeding and post-weaning period of the experiment [a]

	Creep		Post Weaning	
Ingredient, Kg	Soy	Control	Soy	Control
Milo	590.91	568.18	409.09	409.09
Soybean Meal	90.91	---	154.55	---
Cottonseed Meal	---	136.36	---	136.36
Cottonseed Hulls	---	---	272.73	200.00
Alfalfa Pellets	181.82	181.82	22.73	113.64
Molasses	31.82	31.82	40.91	40.91
Premix	22.73	22.73	22.73	22.73

[a], As-fed basis

to be reproductively mature when they have two consecutive blood samples with progesterone concentrations greater than 1 ng/ml of serum (Schneider and Hallford, 1996).

At time of breeding all ewes were placed into a feedlot type pen on the control diet. Rams were introduced to all ewes on August 26, with an average Suffolk ewe age of 6 months and an average Rambouillet ewe age of 6.5 months, for three estrous cycles. After the three cycle breeding period rams were removed. Forty-five days after, ewes were ultrasounded to determine pregnancy status.

STATISTICAL ANALYSIS

Weight and serum data was analyzed using GLM procedures of SAS (Cary, NC) with each individual animal serving as the experimental unit and breed serving as a block. Occurrence of puberty and pregnancy rates were analyzed with the CATMOD procedure of SAS and each animal served as the experimental unit. Treatments were considered to be significantly different when $P \leq 0.05$.

RESULTS AND DISCUSSION

The goals of this research and the results that follow are to determine the effects of soy isoflavones on the early life development in ewe lambs from birth to post weaning and also to identify, if any, differences that soy isoflavones may contribute to the attainment of early puberty in said ewe lambs. Although there are positive and negative effects reported on diets high in soy isoflavones, the concentration of this research is the effects on ewe lambs so growth, performance, and reproductive activity were of importance.

During the study we experienced a record setting drought that forced the culling of some ewe lambs off the study. In addition the control group had one ewe lamb die from unknown reasons. After data collection was finished one ewe in the control reported extremely high estrogen concentrations. The data was analyzed and the ewe was determined to fall outside the upper fence using the box plot analysis discussed in Sullivan et al. (2011). Differences among breed were found not to be significant ($P > 0.05$) so all results are written as control and treatment (Main affect).

Growth and Performance

No differences ($P > 0.05$) were identified in the growth or the performance of the ewe lambs (Table 2). Each treatment reported similar weaning weights, pre-breeding weights, average gain, and average daily gains. Therefore, differences in treatments were related to the ingredient composition of each diet and not to the nutrient value associated with each diet.

Table 2. Growth, performance, and pregnancy differences between control and treatment groups, no statistical differences among treatments (P >0.05)

	Control (CSM)	Treatment (SBM)	SE[a]	P Value
Average Wean Weight, kg	31.24	29.11	1.99	0.32
Average Final Weight, kg	51.65	51.08	1.48	0.77
Average Total Gain, kg	20.71	21.96	1.12	0.41
Average Daily Gain, kg/d	0.21	0.22	0.01	0.41
Conception Rate %	53	47		0.65

[a]. Most conservative standard error

Pregnancy Rate

No statistical differences ($P > 0.05$) in pregnancy rate were found (Table 2). However with such limited size of the treatment and control groups it is suggested that a larger scale of this research might conclude different results. Ede et al. (2011) reported the same results when feeding ewe lambs three different diets with varying levels of soy proteins. However, in his results, he suggested a trend that stated a 17% difference in conception rates between treatments 1 and 3 (treatment 1- no soy, treatment 3- high soy). These treatments diets contain equal amounts of soy isoflavones when compared to diets of this study.

Reproductive Activity

Reproductive activity was measured by serum progesterone showing no difference ($P >0.05$) between treatments. However, toward the end of the collection period some ewes began to show elavated serum progesterone levels suggesting that reproductive maturity, 1ng/ml of progesterone concentration for two consecitive samples, as defined by Schneider and Hallford (1996). This may explain why 50% of the ewes in the experiment bred and conceived with in 45 days of the final blood sample. Another explanation to the apparent increase in reproductive activity would be introduction of the ewes to a ram. The phenomenon is decribed in detail by Bearden et al. (2004), where he explains the effect the the male presence has on the female reproductive cycle and how it has even a greater effect on prepubertal females in their first breeding cycle.

Circulating Estrogen

Bearden et at. (2004) discussed how estrogen levels in the female are a major factor in reproducitve tract growth. Because of this, serum estrogen levels were monitored at weaning to determine differences, if any, the creep diets had from birth to weaning, and then pre-breeding to establish differences the diets had from weaning to breeding. Samples were not taken after ewes were introduced to the ram because all ewes were fed the control diet. This would allow all differences between treatments to be explained by the feeding of different diets pre-breeding. No difference ($P > 0.05$) was found in the estrogen concentration from the samples collected at weaning (Table 3). This is expected even thought the treatment ewes were consuming a diet high in soy isoflavones; they were simply too young for the phytoestrogens to have an effect. However, a difference was reported ($P < 0.05$) in the estrogen concentration of the samples from pre-breeding (Table 3). The findings that the pre-breeding estrogen concentration was different are consistant with Ede et al. (2011) where ewes fed the same diets reported different estriodal concentrations.

Isoflavone Concentrations

Isoflavones are found in any plant product and are defined as a substance that has a hormonal effect on mammalian beings (Ursin et al., 2006). Ede et al. (2011) reported that the three main isoflavones we are dealling with are daidzin, genistin, and glycitin. The activation rates of the phytoestrogens reported are 477, 666, and 150 mg/g for daidzin, genistin, and glycitin. The control group diet contained no soy protein there for intake was 0 mg/d. The

treatment group ended the experiment with an average daily feed intake of 1.79 kg/day, so each ewe ingested an average of 298.8 g of soybean meal/day. Therefore, intake of

Table 3. Serum estrogen concentrations of ewe lambs from control and treatments groups

	Treatments			
	Control (CSM)	Soy (SBM)	SE[a]	P-value
Weaning E2 Concentration (ng/ml)	2.00	3.13	1.24	0.48
Pre-Breeding E2 Concentration (ng/ml)	4.30	14.31	3.69	0.04

[a]. Most conservative standard error

individual isoflavones was 142527.6 mg/d of daidzin, 199000.8 mg/d of genistein, and 44820.0 mg/d of glycitein (Table 4).

Table 4. Soy isoflavone intake of ewes consuming diets based on an as fed basis of 3.5% of end body weight

	Daidzein	Genistein	Glycitein	Totals
Isoflavone Levels, mg/g of SBM	477.0	666.0	150.0	1293.0
Control mg/d, (0.0 g of SBM/d)	0.0	0.0	0.0	0.0
Treatment mg/d, (298.8 g of SBM/d)	142527.6	199000.8	44820.0	386348.4

IMPLICATIONS

The results of this study suggest that feeding diets high in soy isoflavones will have an effect on circulating estrogen levels in ewe lambs. By increasing circulating estrogen levels in ewe lambs it is possible to influence attainment of puberty. This, with more research, could be proven to increase ewe lamb conception rates. In the instance of a small producer it may not have as much of an impact as when considering a large scale operation. A 10% increase of ewe lamb conception has the possibility of making a large impact on the commercial breeder. This increase of ewe lamb conception will not only increase first year lamb crop, but will have a positive influence on said ewes lifetime production.

One major environmental influence on this research was the record setting drought that was experienced during the summer of 2011. In conjunction with the drought we experienced record setting temperatures for the year. In 2011, we experienced the most days over 37.8^{o}C with 100d, and the most days over 40.6^{o}C with 37d (National Weather Service). The summer was also recorded as the 3rd driest year on record with an annual precipitation of 23.4 cm (National Weather Service).

Heat stress in the female is a well-researched and understood topic in livestock. Heat stress in the female can cause many different adverse effects. Heat stress will delay puberty in both males and females (Bearden et al., 2004). This might be the case in the ewes on the experiment even though we are trying to increase estrogen levels chemically. Natural response to the environment could easily overpower the chemical response to the phytoestrogens. Further research on the subject might yield different results during a year with average precipitation and temperature.

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